BIOCHEMICAL FUEL CELLS

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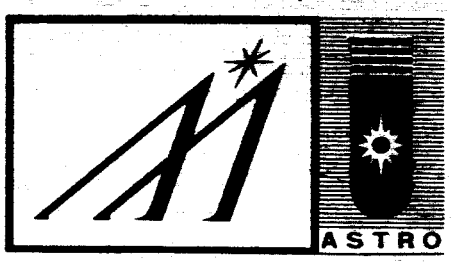

THE MARQUARDT CORPORATION

Fifth Quarterly Progress Report

1 May 1964 through 31 July 1964

STUDY OF BIOCHEMICAL FUEL CELLS

Contract NASw-654

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I. SUMMARY

During the previous quarter, some emphasis has been placed upon the study of the use of electrochemical energy in degrading human waste, in addition to maximizing the electrochemical power output of human waste as a biofuel.

It has been found that there was essentially no difference in the amount of electrochemical energy obtainable from human waste under either aerobic or anaerobic conditions.

The non-biological cathode reactions, involving bubbling with gaseous oxygen in a salt solution containing 2½% by weight of sodium chloride and 2½% potassium chloride, have been found to be rate determining to the extent of about 8:1.

Some rapidly growing microorganisms indigenous to human waste have been isolated and then added to non-sterile human waste, but without any appreciable change in the electrochemical properties of the waste.

Fresh urine was compared with frozen and reheated urine as a biofuel; there was no noticeable difference in electrochemical energy output, though it had previously been found that fresh urine was more electrolytically conductive.

Reproducibility studies have been continued, to verify the limited amount of variability in the techniques and materials used in the experimental systems during the extended period of this contract (approximately 18 months). The results of these experiments were satisfactory.

Electrochemical energy is being used to degrade human waste, the input of current being controlled with a galvanostat. The human waste had been frozen and thawed (the feces were obtained from a special low-cellulose diet), and was used alone as well as with activated sludge obtained from a municipal sewage treating plant (courtesy Mr. Joseph Nagano, Hyperion Sewage Works, Playa del Rey, California). The purpose of these experiments is to determine whether electrochemical energy can be employed in degrading human waste, either to increase the reaction rates or produce less toxic products than conventional sewage disposal techniques.

Author

II. INTRODUCTION

This report covers the progress attained during the Fifth Quarter, 1 May 1964 through 31 July 1964, in the Study of Biochemical Fuel Cells, Contract No. NASw-654. The purpose of this program is to conduct empirical studies on biochemical fuel cells for producing electrical energy through degradation of human waste.

III. DISCUSSION

A. EFFECT OF AEROBIC CONDITIONS IN THE FUEL-ANOLYTE

The final phase of this contract is to be devoted to some extent to studies of the use of electrochemical energy in degrading human waste, in addition to maximizing the electrochemical power output of human waste as a biofuel as has been done in the earlier phases of this program.

In an attempt to facilitate the degradation process, an experiment was conducted to determine the effect of aerobic conditions upon the electrochemical power output of the biofuel cell, in contrast to the anaerobic conditions used in the past. The conditions of the experiment were generally the same as those which were selected in the early phases of this program in an effort to standardize the experiments and minimize the variables. These conditions are summarized in Table I. Two cells were assembled. One was a control, assembled as described in Table I; the other was a test cell, in which oxygen instead of helium was bubbled through the fuel-anolyte. The fuel-anolytes and catholytes used in both cells came from the same mixtures. The results of the experiment (Experiment 67) are summarized in Table II.

It was found that there was essentially no difference in maintaining the fuel-anolyte under aerobic as contrasted to anaerobic conditions. The open-circuit anodic potentials and short-circuit current densities of both systems improved during the first 100 hours of the test, due to metabolic reactions. However, at any given time there was only a negligible difference in open-circuit anodic potentials and short-circuit current densities between the aerobic and anaerobic systems.

B. DETERMINATION OF LIMITING CURRENT DENSITIES

In the experiments conducted previously, the anode and cathode areas have been the same, for simplicity. However, the determination of limitations of current densities is a matter of fundamental importance in electrochemical studies. Therefore, experiments were conducted in which the ratios of cathode-to-anode electrode areas were varied, and it was found that the cathode reaction was rate-limiting. The experimental conditions were essentially the same as those described in Table I, except that the anode area was varied; cathode-to-anode area ratios of 2:1, 4:1, and 8:1 were employed.

The results of the experiments (Experiments 68 and 75) are summarized in Table II.

C. ELECTROCHEMICAL ACTIVITY OF MICROORGANISMS ISOLATED FROM HUMAN WASTE

Some rapidly growing microorganisms indigenous to human waste have been isolated, in an effort to determine whether the addition of these selected strains can improve the electrochemical activity of the composite mixture used in most of the previous studies. The electrochemical behavior of selected strains may be better or worse than that of the mixture, depending upon whether metabolic products released in the mixture are inhibitory or enhancing to the other reactions.

The experimental conditions were essentially the same as those described in Table I. Four nonflow cells were assembled: One was a control cell, assembled exactly as described in Table I; the second cell contained a portion of a strain of bacteria (identified as culture #2) isolated from human waste on agar; the third contained another strain (culture L) isolated from the waste in similar fashion; and the fourth contained a strain (No. 11) which had been obtained as a contaminant from an H-cell that had been given a routine test for sterility, after the usual cleaning process following use of the H-cell as a biofuel cell. The contaminant strain (No. 11) grew profusely in trypticase soy agar medium, and was considered to be a logical choice for metabolic and possibly electrochemical reaction in the fuel cell.

In each of the three cells described above, where strains of isolated bacteria were added to the fuel-anolyte, the addition was made to mixtures of indigenous microorganisms rather than to sterilized waste mixtures. The methods employed in growing the cultures are described in the Appendix.

It was found that the anodic open-circuit potentials of the four cells were essentially the same, within approximately 25 millivolts throughout most of the experiment. It was also noticed that the anodic open-circuit potentials of all four cells began to change significantly after 35 hours, and went from approximately -460 millivolts to -650 millivolts (saturated calomel reference) at the end of an additional 40 hours, where they remained essentially constant for the remaining 48 hours of the experiment. These curves are shown in Figure 1 of the Appendix.

Polarization and power data obtained with strain No. 11 (i.e., the contaminant from the H-cell) gave an unusually high current density and anodic power density (see Experiment 69, Table II of the Appendix). Although the probable sources of error were checked (e.g., contaminated electrodes or gas manifolds, etc.), no error was found in the data.

The experiment was repeated for verification (see Experiment 73, Table II of the Appendix). Again, four cells (nonflow type) were assembled. One was a control cell, assembled under the conditions described in Table I; the second cell contained bacteria strain No. 2; the third sample contained a new sample of bacteria strain No. 11; and the fourth contained bacteria strain No. 11 from the previous experiment, added to fresh urine-feces mixture (10% old fuel-anolyte mixture and 90% new mixture). The repeat experiment did not verify the previous high values of potential.

It was noted, however, that the phenomenon of the agreement of the anodic open-circuit potentials with time for the four cells was verified, even during periods of rapid changes of potential. The anodic open-circuit potentials of the four cells were still nearly the same at any given time (though not quite as good as the agreement in the previous experiment), and the potentials of all four cells simultaneously began to change after approximately 35 hours of the test, changed about 175-200 millivolts over the next 15 hours, and remained essentially constant over the remaining 100 hours of the experiment.

The relatively large changes in potential doubtless are caused by metabolic reactions. These experiments have shown that the rates and extent of the metabolic reactions of indigenous microorganisms were not significantly affected by the addition of selected microorganisms to the indigenous system.

D. COMPARISON OF FRESH URINE AND FROZEN-REHEATED URINE AS A BIOFUEL

The human urine used in these investigations has usually been frozen and later reheated, for simplicity in procurement. However, after freezing, some of the solids do not go back into solution, even after heating to 120°F; heating to higher temperatures has been avoided because of the possibility of degrading components of the waste.

Measurements were made earlier in this program of the effects of these solids upon the electrolytic conductivity of the urine. It was found that the electrolytic conductivity of the urine was significantly less when these solids were formed and were not redissolved.

An experiment was run to determine whether removing these solids from the urine also affected the electrochemical power output of the fuel. There seemed to be little difference in the power output of the two types of urine (fresh or frozen and thawed). The data are reported in Table II (Experiment 70). The power output from the frozen and thawed urine was lower than that obtained previously from similar systems. The data obtained with the fresh urine seemed to have been obtained at an

inopportune time, since the anodic open-circuit potential at that time was -0.450 volt and later (after 48 hours) had attained -0.650 volt.

The previous procedure of freezing and thawing the urine, for convenience, will be continued.

E. REPRODUCIBILITY STUDY

A standard reproducibility run (Experiment 72) was made with the flowing system. The experimental conditions were the same as those described in Table I.

Two cells were assembled in parallel, and the statictical data covering the entire 124 hours and the final 64 hours of the experiment are summarized below.

	Entire 124 Hours	Final 64 Hours
Mean of Maximum Difference of Potentials (millivolt)	64.1	28.6
Standard Deviation	46.7	35.5
Variance	2190	1275

F. USE OF ELECTROCHEMICAL ENERGY FOR DEGRADING HUMAN WASTE

Two nonflow cells were assembled, under essentially the same conditions described in Table I, except that the fuel-anolyte of one cell was bubbled with oxygen instead of helium.

The cells were connected in series to a galvanostat and a current of 10-14 microamperes was put into the cell over a period of 15 days, for a total of 14.14 coulombs. The area of each electrode was 1 sq. in.

The fuel-anolytes of these cells (Experiment 74) will be lyophilized and combusted in the oxygen bomb calorimeter to determine the effect of using electrochemical energy in degrading human waste under both aerobic and anaerobic conditions.

If it is found that electrochemical energy has been effective in degrading the waste, more extensive studies will be made (such as chemical analyses of the products) to determine the specific nature of the degradation process.

G. SEPARATION OF CHEMICAL-BIOCHEMICAL FROM ELECTROCHEMICAL FUEL CELL REACTIONS

A series of experiments has been conducted during recent months to separate chemical-biochemical reactions (i.e., those that occur away from the proximity of an electrode) from electrochemical reactions that occur only at the electrode surface.

The method involves the use of flow and non-flow systems under the experimental conditions described in Table I, comparing the variation in electrochemical properties with time. In the non-flow system, the total volume of fuel-anolyte mixture is confined near the electrode and is accessible to the electrode, whereas in the flow system only a small portion of the fuel-anolyte is actually in the fuel cell at a given time and the remainder is in the reservoir. Because of the agitation provided in both systems, the diffusion rates are probably high enough that they are not the limiting steps in the reaction. In order to permit electrochemical reactions to occur, a current is withdrawn from the flow and nonflow cells and is maintained constant by a galvanostat; the cells are connected in series so that the same current is withdrawn from both cells.

In the previous experiments, there has been no correlation between the potentials of the flow and nonflow systems, on either a total time or cell time basis. Therefore, an experiment is now being conducted in which the ratio of electrode area to fuel-anolyte volume is the same in both systems (Experiment 81). This will permit proportionate electrochemical reaction to occur.

H. ELECTROCHEMICAL ACTIVITY OF ESCHERICHIA COLI ON AN AMINO ACID

A limited effort is being expended in determining some of the reaction mechanisms associated with chemical and electrochemical degradation of human waste. The basic plan is to determine rather simply whether one or two of the many types of microorganisms indigenous to human waste may be responsible for producing most of the electrochemical power that is obtainable from human waste, by preferential metabolic reactions involving one or two of the indigenous chemical compounds.

A list of chemical compounds indigenous to human feces was presented in the thirteenth monthly report. It was stated that several general types of chemical compounds (carbohydrates, lipids, and proteins) are present in human waste.

The initial study involves determining the electrochemical energy output caused by the metabolic reaction of one of the types of indigenous microorganisms (Escherichia coli) on one of the amino acids that constitute the protein content of human feces.

Two experiments are being conducted under the conditions described in Table I for the non-flow system, except that the fuel-anolyte contains only an amino acid (arginine in Experiment 77, threonine in Experiment 79) in sterile, deionized water. The length of time required for this experiment is expected to be very short (e.g., a few hours) because it is recognized that the small amount of amino acid being used in this experiment (approximately 1/6 oz.) will be quickly consumed, and further that a medium containing only water and one additional chemical compound (the amino acid) is not a satisfactory nutrient for continued microbiological metabolism.

I. ELECTROCHEMICAL DEGRADATION OF ACTIVATED SLUDGE

A sample of activated sludge was obtained from the local sewage treating plant (courtesy Mr. Joseph Nagano, Hyperion Sewage Works, Playa del Rey, California).

This material is being maintained in an aerated, viable condition by bubbling with gaseous oxygen and by periodically adding fresh human waste to the sludge.

The sludge is being used under experimental conditions similar to those described in Table I for the non-flow system, except that the activated sludge has been added to the urine-feces mixture in a final volume ratio of 1 volume of activated sludge to 9 volumes of the usual urine-feces mixture, and the fuel-anolyte will be maintained aerated (Experiment 80).

IV. FUTURE WORK

This program is in its final month, and no new studies will be initiated. The time will be spent in making final reproducibility runs, in making bomb calorimetric measurements of samples of human waste that have been degraded for varying periods of time, in reviewing the data to determine whether some item should be repeated for verification and confirmation, and in final experiments employing the optimization of sewage (waste degradation) techniques.

VI. APPENDIX

A. DESCRIPTION OF METHODS USED IN ISOLATING MICROORGANISMS FROM HUMAN WASTE

Strain L:

A mixture of non-sterile, human urine and feces was prepared, containing 30 grams feces in 100 milliliters of urine. The mixture was centrifuged to remove solids.

Agar was prepared in 0.9 weight percent sodium chloride solution, and then sterilized. The agar mixture was added to the supernatant liquid obtained by centrifuging the human waste and then poured on plates. The agar plates were then incubated at 37°C for 24 hours.

The colonies which grew on agar were streaked on trypticase soy agar incubated at 37°C for 24 hours, then refrigerated.

Strain No. 2:

Plates were prepared by mixing equal volumes of non-sterile urine or urine-feces with 3 weight percent agar containing 1 weight percent sodium chloride. Half of the urine plates were streaked with a non-sterile emulsion of feces. Therefore, there were three kinds of agar plates; one contained urine-feces mixture, another contained feces streaked on urine, and the third contained urine only.

These plates were allowed to stand at room temperature for four days, half of the plates under aerobic conditions and half under anaerobic conditions. Then colonies were taken from the plates, added to trypticase soy broth, and stained.

The strain designated as No. 2 was obtained from a urine-agar plate streaked with feces and maintained aerobically. A large colony grew, which resembled Bacillus and had large, Gram-positive rods in chains.

Slants were prepared of this organism, and when streaked on sterile urine and urine-feces plates, growth was visible after 24 hours. Growth was better on the urine-feces plates.

Strain No. 11:

A sample of contaminant was removed from glass, H-cells which had supposedly been sterilized. The contaminant was transferred to trypticase soy agar, and incubated at 37°C. The "contaminant" grew and was pink in color, and was composed of Gram-positive large rods and ovals.

VARIATION OF OPEN-CIRCUIT POTENTIAL WITH TIME
NONSTERILE HUMAN WASTE WITH AND WITHOUT ADDED MICROORGANISMS
ISOLATED FROM HUMAN WASTE

EXPERIMENTAL CONDITIONS ARE DESCRIBED IN TABLE I
METHODS USED IN ISOLATING THE MICROORGANISMS ARE
DESCRIBED IN APPENDIX

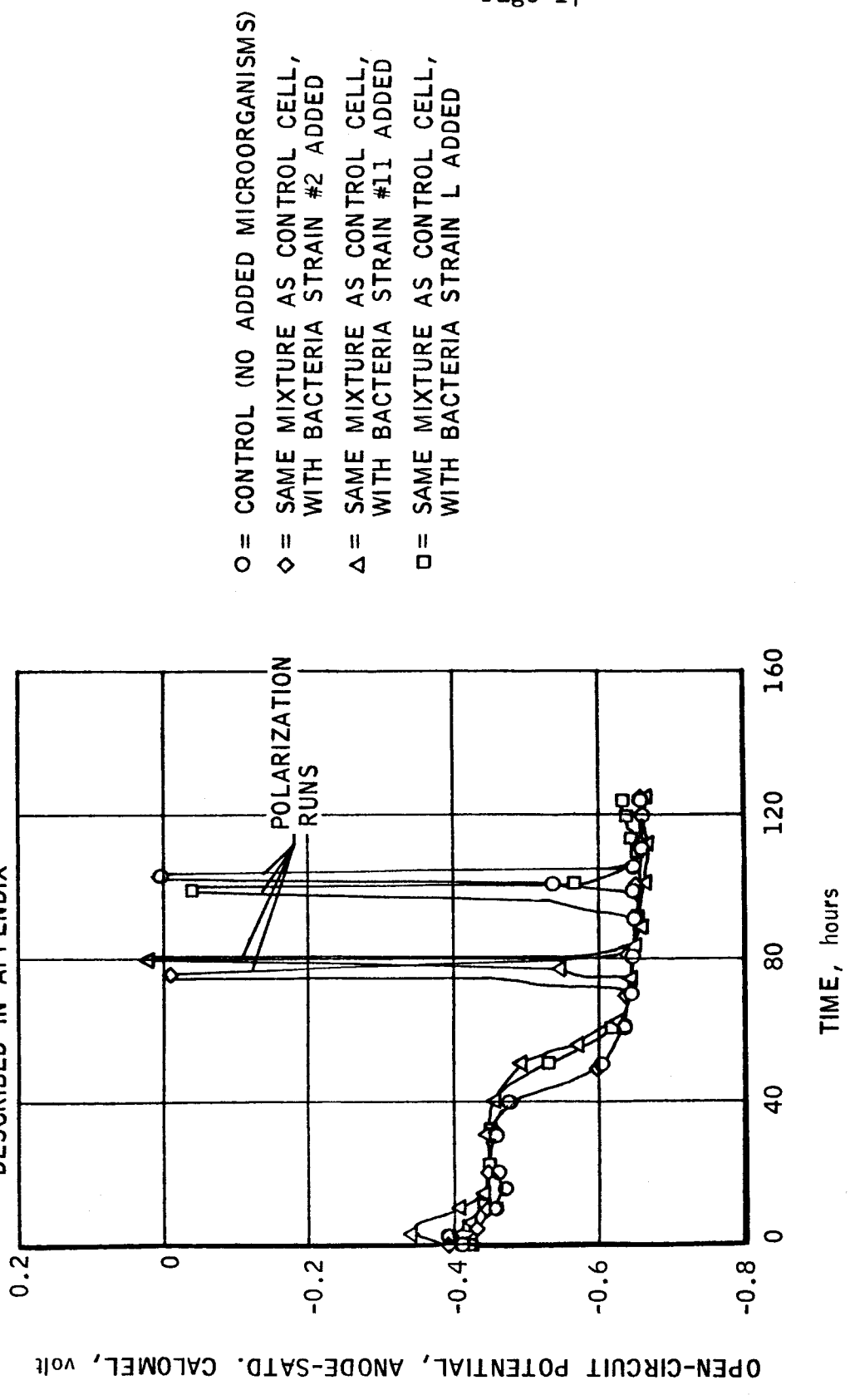

FIGURE 1

TABLE I

EXPERIMENTAL CONDITIONS

BIOCHEMICAL FUEL CELL

FLOW SYSTEM

Cell: Plastic (Lucite)

Electrodes: Platinized screen (90% Pt - 10% Rh), 80 mesh, 0.003 in. diameter wire, 2-1/8 in. clear diameter, 3.54 sq. in. geometric area.

Separator: Cellulose acetate (Sargent S-14825, 0.001 in. thick).

O-Rings: Silicone (Dow Corning S-7180).

Catholyte: 5 wt. % NaCl - 5% KCl in sterile, deionized water; non-biological; bubbled with purified, gaseous oxygen.

Waterproof and Chemically Resistant Paint: Temprotec TP 220 Red (Ryan Herco Products Corp., Burbank, Calif.)

Fuel-Anolyte: 30 gms. non-sterile, human urine (frozen and reheated to 120°F). Feces was obtained from volunteers on a low cellulose diet, and was frozen immediately after collection. Homogenized in Osterizer Deluxe (John Oster Mfg. Co., Milwaukee). Bubbled with helium.

NON-FLOW SYSTEM

Cell: Glass, H-Shape, O-Ring type

Electrodes: Platinized platinum foil, 1 sq. in. area (non-opposing faces coated with waterproof and chemically resistant paint).

Separator, O-Rings, Catholyte, Fuel-Anolyte, and Waterproof and Chemically Resistant Paint: Same as for flow system.

TABLE II

POLARIZATION AND POWER DATA

Run No.	67	67	67	67	67	67
Described in Section	IIIA	IIIA	IIIA	IIIA	IIIA	IIIA
Peak Anodic Power Density (mw./sq.ft.)	0.55	0.55	3.7	3.8	3.5	3.1
Peak Total Power Density (mw./sq.ft.)	0.75	0.95	5.0	4.8	4.95	4.0
Short Circuit Current Density (ma./sq.ft.)	19	22	41	44	42	37
Open-Circuit Anodic Potential at Time of Polarization Study (volt)	-0.200	-0.425	-0.390	-0.502	-0.390	-0.480
Best Anodic Open-Circuit Potential (volt)	-0.460	-0.525	-0.460	-0.525	-0.460	-0.525
pH, Initial	8.5	8.5	8.5	8.5	8.5	8.5
pH, Final	8.8	8.7	8.8	8.7	8.8	8.7
Duration of Test (hours)	167	167	167	167	167	167
Elapsed Time at Polarization (hours)	29	31	101	104	149	152
Distinguishing Variable	Oxygen	Helium	Oxygen	Helium	Oxygen	Helium

TABLE II (Continued)

POLARIZATION AND POWER DATA

Run No.	68	68
Described in Section	IIIB	IIIB
Peak Anodic Power Density (mw//sq. ft.)	0.5	0.9
Peak Total Power Density (mw./sq. ft.)	0.9	1.3
Short Circuit Current Density (ma./sq.ft.)	23	25
Open-Circuit Anodic Potential at Time of Polarization Study (volt)	-0.425	-0.372
Best Anodic Open-Circuit Potential (volt)	-0.675	-0.615
pH, Initial	8.5	8.5
pH, Final	8.8	8.8
Duration of Test (hours)	86	86
Distinguishing Variable	1/2 anode	1/4 anode

TABLE II (Continued)

POLARIZATION AND POWER DATA

Run No.	69	69	69	69	70	70
Described in Section	IIIC	IIIC	IIIC	IIIC	IIID	IIID
Peak Anodic Power Density (mw./sq.ft.)	1.55	7.6	*	*	0.6	**
Peak Total Power Density (mw./sq.ft.)	2.32	10.8	*	*	0.8	**
Short Circuit Current Density (ma./sq.ft.)	29	90	*	*	14	**
Open-Circuit Anodic Potential at Time of Polarization Study (volt)	-0.638	-0.670	-0.652	-0.640	-0.450	-0.490
Best Anodic Open-Circuit Potential (volt)	-0.665	-0.670	-0.662	-0.665	-0.643	-0.613
pH, Initial	7.8	7.8	7.8	7.8	8.65	8.3
pH, Final	-	-	-	8.5	8.85	8.65
Duration of Test (hours)	122	122	122	122	150	150
Distinguishing Variable	Added Strain #2	Added Strain L	Control	Added Strain #11	Fresh Urine	Frozen-Thawed Urine

* Erroneously high values, reason unknown
** Erroneously low values, reason unknown

TABLE II (Continued)

POLARIZATION AND POWER DATA

Experiment No.	73	73	73	75
Described in Section	IIIC	IIIC	IIIC	IIIB
Peak Anodic Power Density (mw/sq ft)	0.9	0.69	0.98	0.22
Peak Total Power Density (mw/sq ft)	1.35	0.84	1.24	0.22
Short Circuit Current Density (ma/sq ft)	15	12	14	5
Open-Circuit Anodic Potential at Time of Polarization Study (volt)	-0.650	-0.472	-0.527	-0.501
Best Anodic Open Circuit Potential (volt)	-0.650	-0.615	-0.540	-0.580
pH, Initial	8.3	8.3	8.3	7.3
pH, Final	8.9	8.9	8.6	8.3
Duration of Test (hours)	186	186	186	170
Distinguishing Variable	Bacteria 2	Bacteria 11 from Expt. 69	New Bacteria 11	-